Chemistry and Imaging for Martian and Earth Soils

A wet chemistry suite with specific life-detection capability

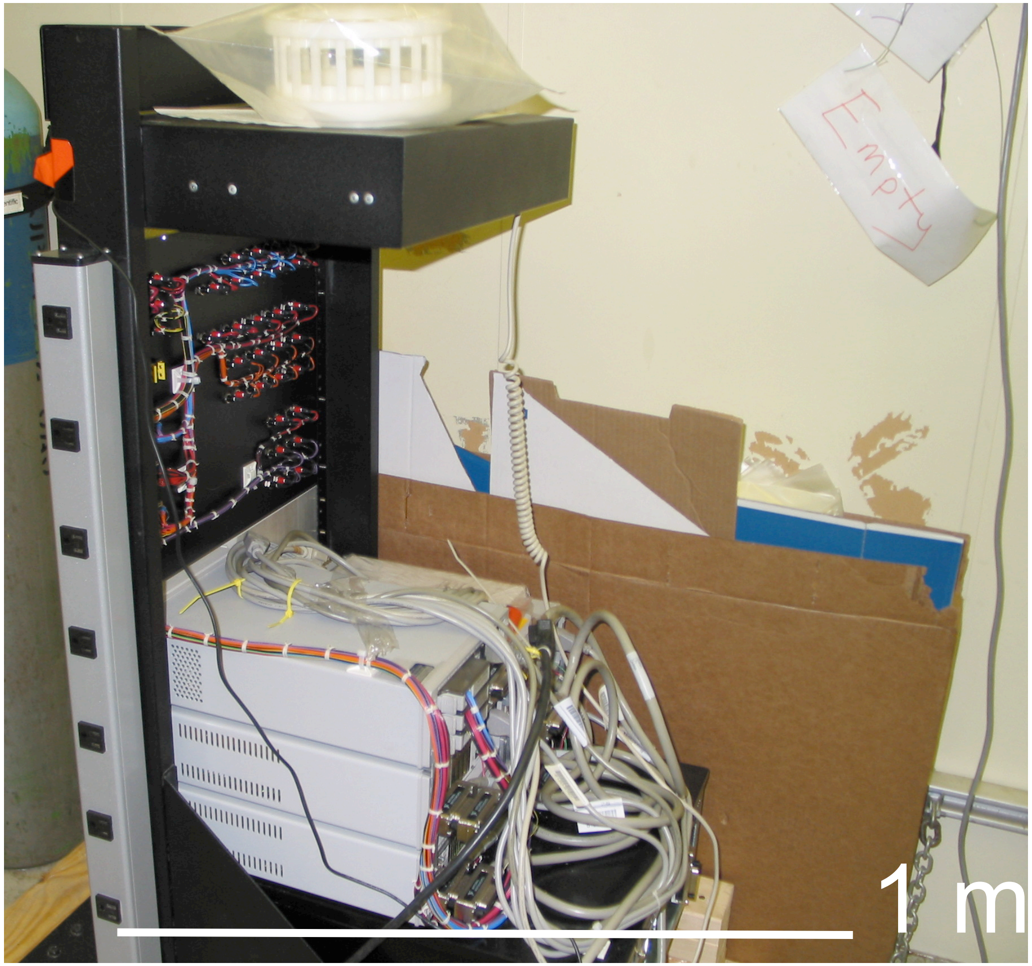
General purpose

- To use the Robotic Chemistry Lab (RCAL) from Starsys Research to create a wet chemistry instrument to study Martian mineralogy, past history, and biosignatures
- Biosignatures may be microscopic (bacteria or fragments of bacteria) or macroscopic (ionic gradients caused by the presence of layers of microorganisms)

Three-pronged approach

1. Development of the RCAL: how many ISEs can reasonably be included? How many other sensors should be included and what should they be? Does the can need to be redesigned to hold them?
2. Basic mineralogy and soil chemistry to identify minerals and ionic gradients that can act as biosignatures (scale: 1 cm - tens of m)
3. Development of an *in situ* fluorescent labeling and imaging scheme

RCAL: delivered 12/03

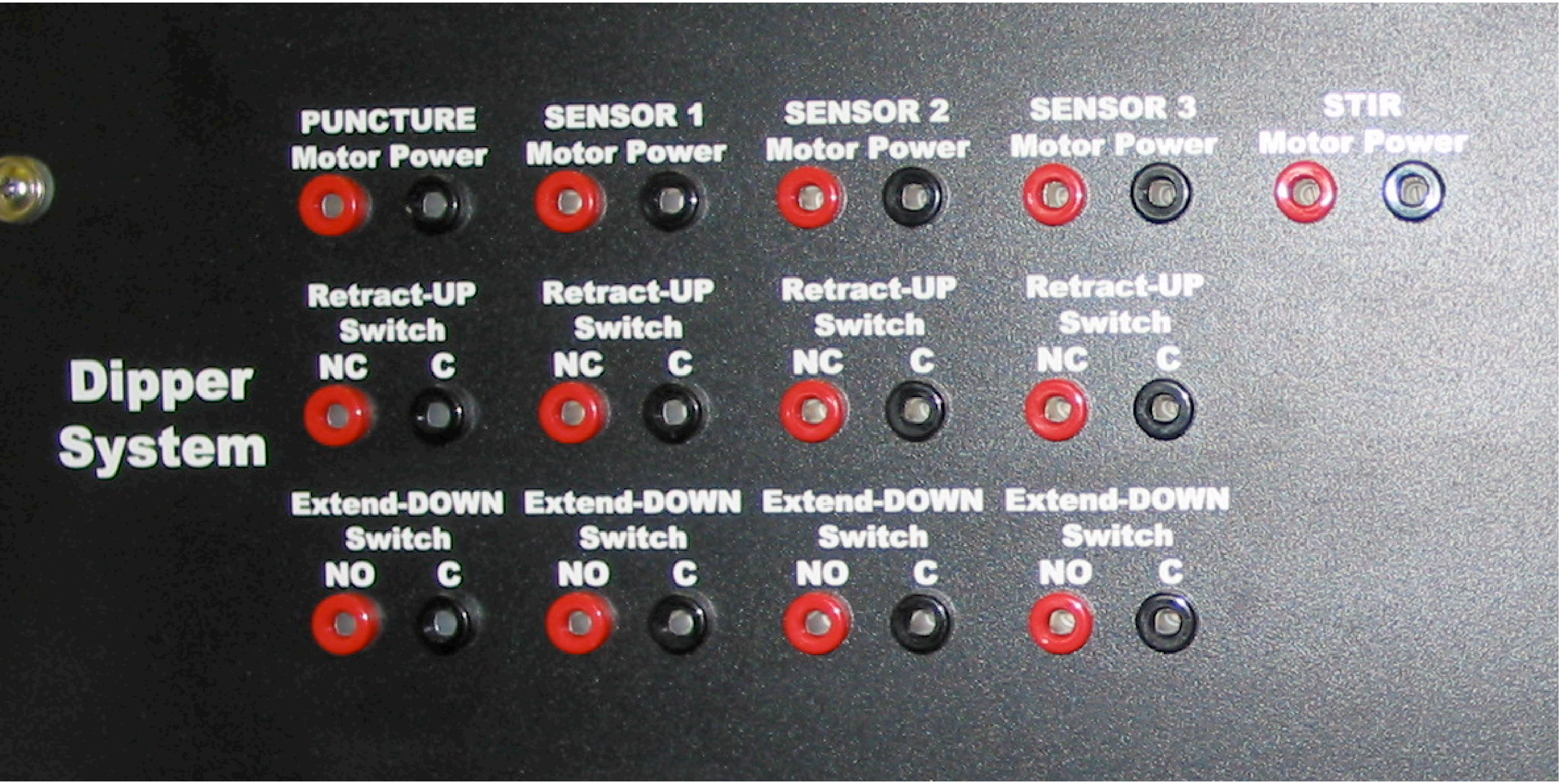


The bulk of the instrument is the electronics control system and its associated computers

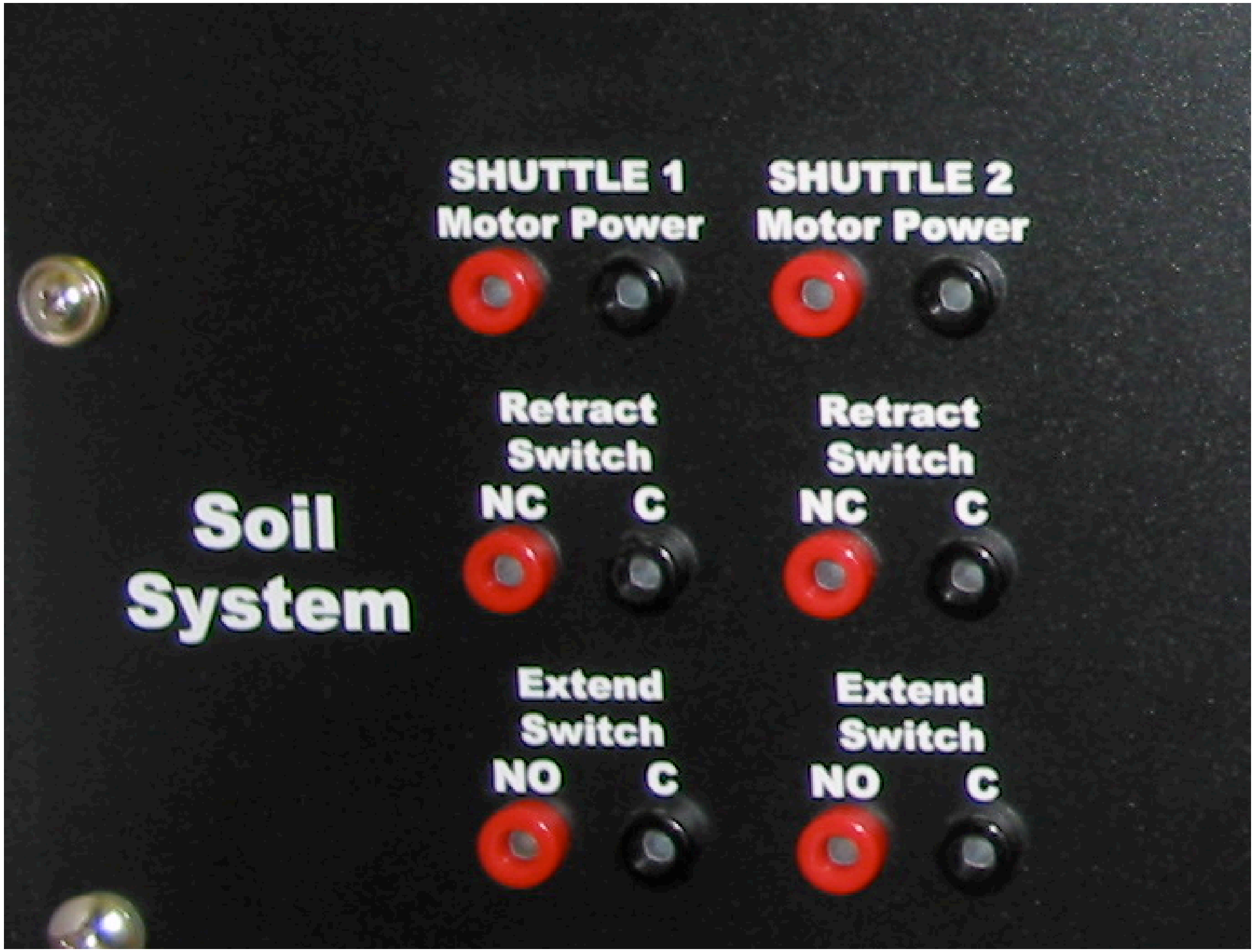
A flight version would require miniaturized electronics!

Control panels

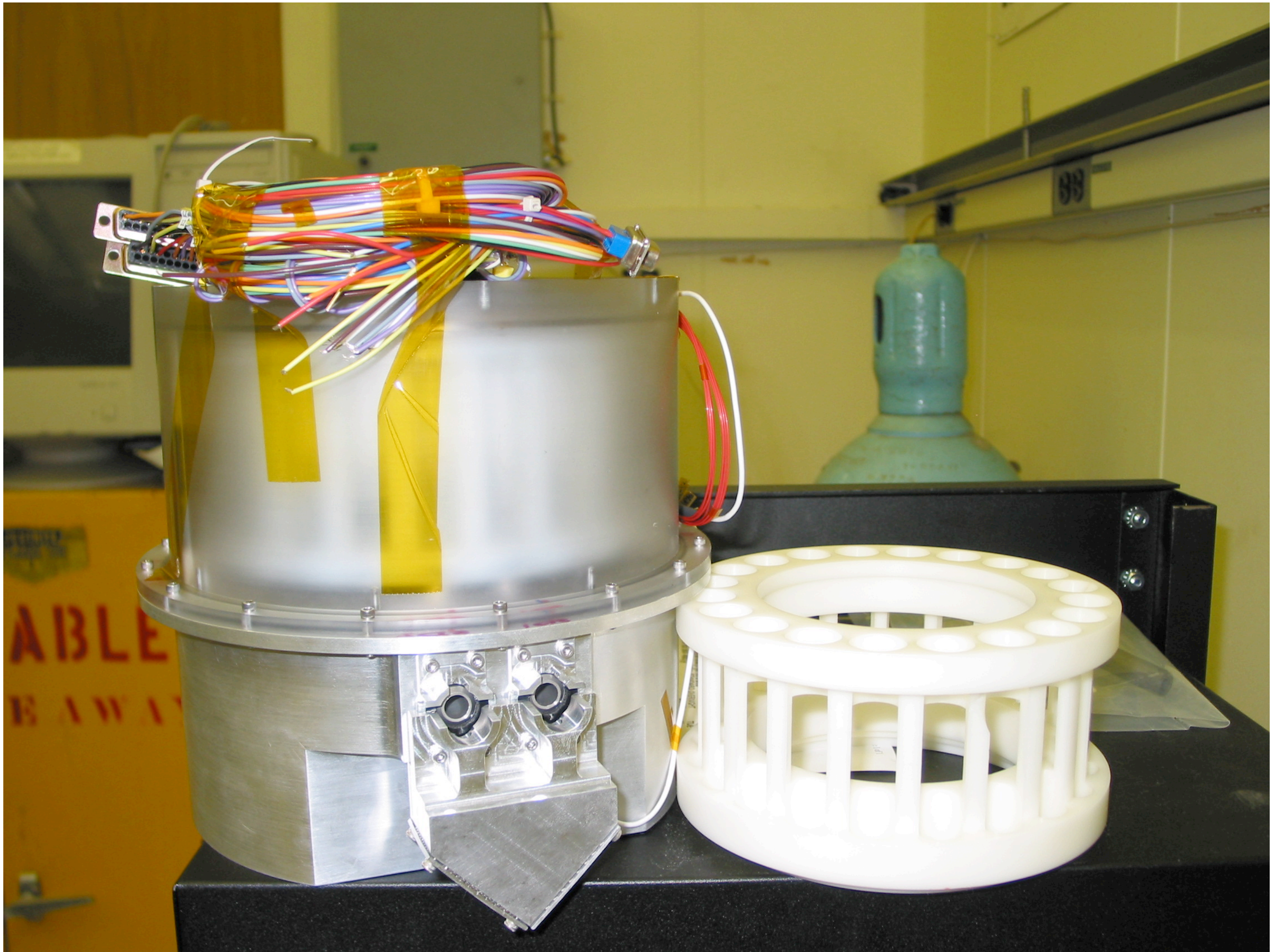
3 sensors + stir



Automated soil drawer



Carousel and outer can



Issues

- A better soil delivery system is absolutely required; “throwing” soil at hopper is inefficient and delivers the particles least likely to contain organics
- 3 sensors isn’t many; wise choice is required
- Size of can may have to be enlarged somewhat to contain instruments

Desired instrumentation

- ISEs
- Pentrode
- Fluorescence/absorbance spectrometer
- Camera
- Microscope

Proposed arrangement

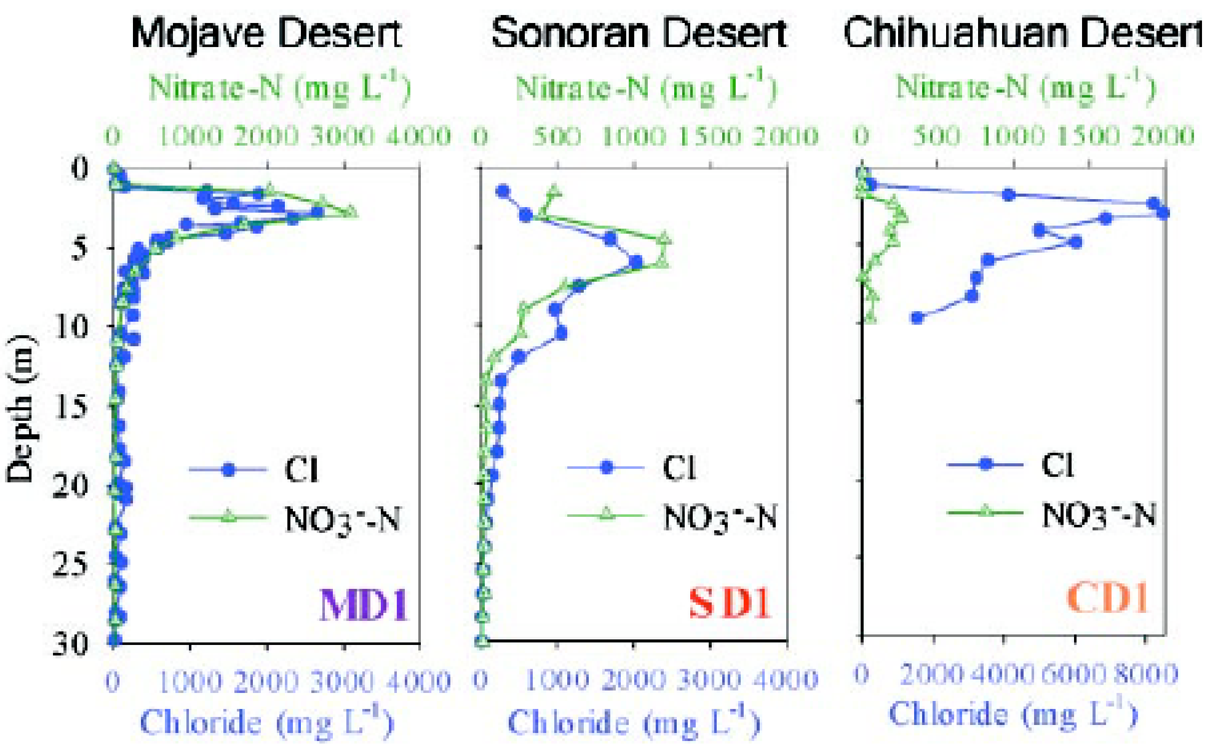
2. Chemistry with ion selective electrodes (ISEs) and colorimetric reagents

- Using ISEs
- An example system: “peds” from desert pavement
- Results of water extraction
- Other extractions
- Other ions we would like to measure: phosphate, magnesium, Fe(II) and colorimetric agents
- Problems and future work

An interesting aside: ions as a meter-scale biosignature

A Reservoir of Nitrate Beneath Desert Soils

Michelle A. Walvoord,1
Fred M. Phillips,2 Davi
A. Stonestrom,3
R. Dave Evans,4 Pete
C. Hartsough,5,6 Bren
D. Newman,7
Robert G. Striegl1,
Science 302: 1021-10
(2003)



What can we measure with ISEs and what can it tell us?

All off-the-shelf sensors from ThermoOrion: pH, conductivity, redox potential, permanganate, nitrate, ammonium, Cd^{++}, dissolved oxygen and CO_2, Br^-, Ca^{++}, I^-, Cl^-, K^+, Ag^+-S^{--}, Mg^{++}

Typical extraction method uses dH_2O at a ratio of 10:1 v/w liquid: soil. Stir for1-24 hr and filter particulates. Most soils become turbid under these conditions

Typical amount of soil needed is 500 mg -20 g

Sample systems

- Pasadena arroyo: chaparral, superfund site (possible permanganate?)
- Desert "peds" from Mojave (have ICP results with which to compare)

Note "rind"

Observed values of water-soluble ions for two soil samples (μg/mL)

	Ca	Mg	Na	K	NO3	S	Cl
Arroyo	3.0	3.0	1.0	2.5	3.0*	5.0	1.0
Desert	1.0	1.2	2.0	1.0	0.3	2.0	5.0

* Value disagrees with ICP

Not detected: Cd, permanganate

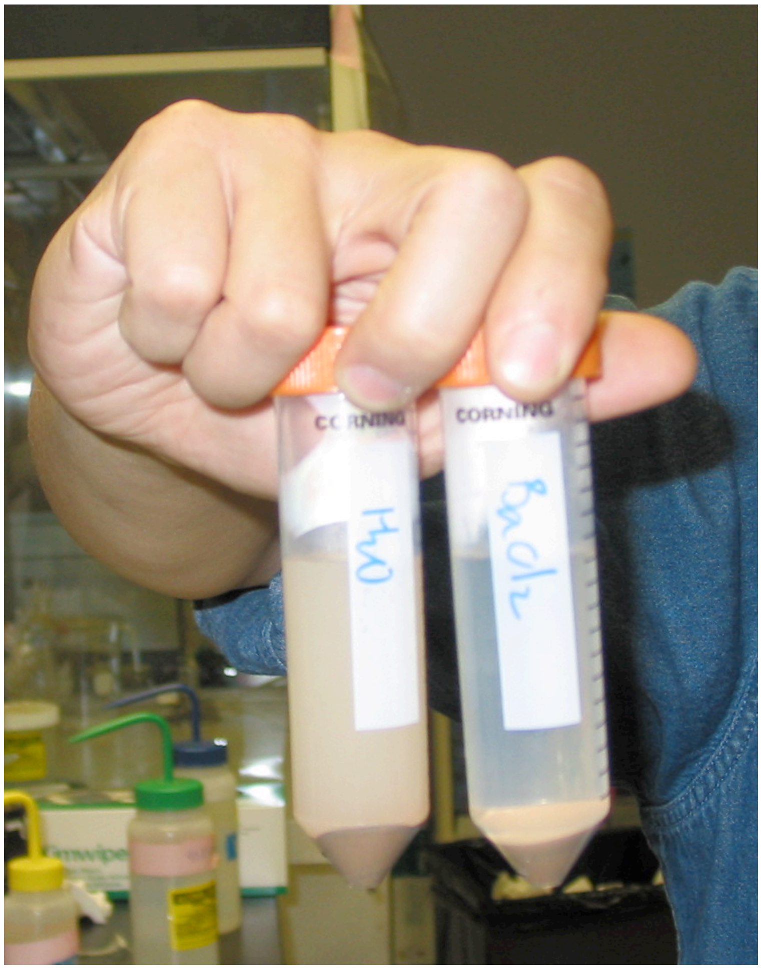
Typical variability/error

- <1 % for filtrates
- Greater if particles are present
- Greatest for nitrate electrode
- Most failed recordings are not inaccurate, but fail to give a value at all

General observations

- At least 10 g of soil is ideal amount
- Particulates must be filtered: adhesion of particulates to ISE surface causes faulty readings (electrostatic adhesion?)
- Desert “peds” must be crushed to obtain values of anything besides Na, Cl
- Results are not always identical for different water: soil ratios! Consistency is key
- Nitrate often seems to run high
- High concentration of iron in soils leads to errors esp. for Cd and the halogens; large concentrations of soluble salts also interfere

Other types of extraction

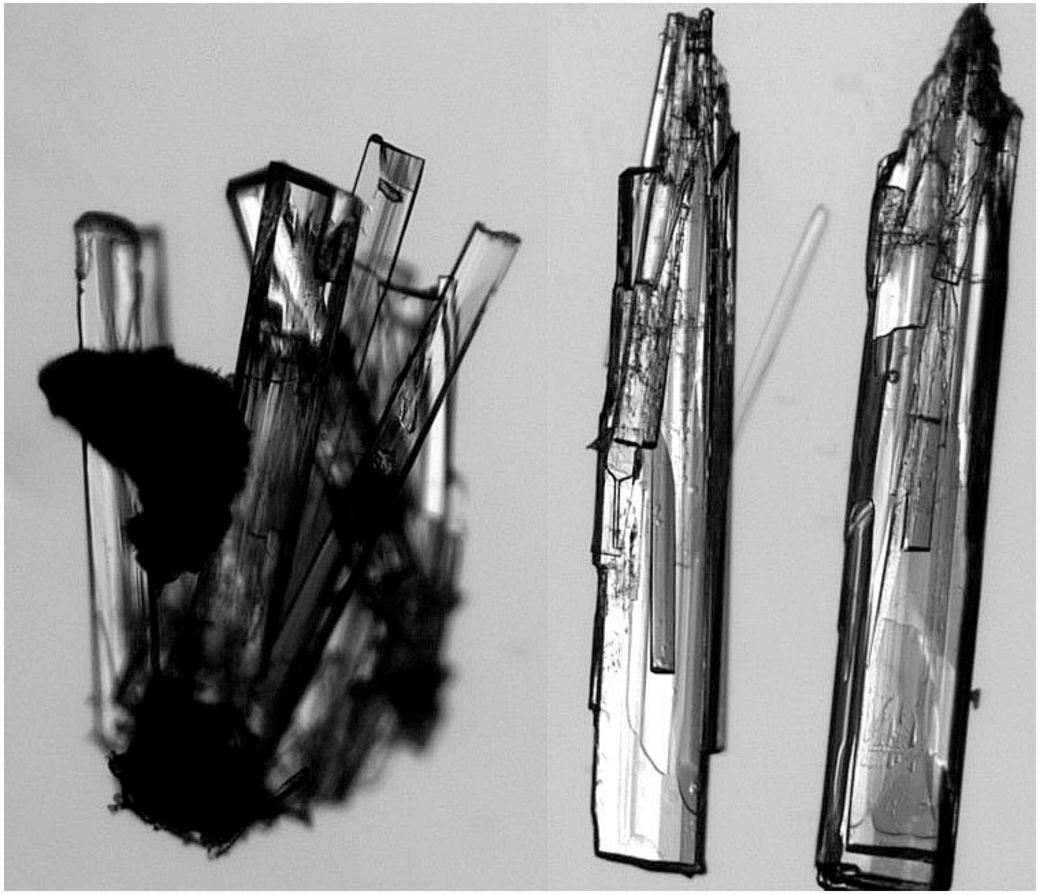


- Exchangeable ions measured by $BaCl_2$ extraction
- Anomalously low values for desert peds are seen!
- Iron oxides can be extracted with acid (nitric/HCl)

Additions to ISEs: colorimetric reagents for analytes present in large concentrations

- There are colorimetric phosphate assays; however, usually several processing steps are required. We will perform experiments to see whether this can be done in the RCAL
- Bipyridyl is a colorimetric indicator for Fe(II) and is extremely valuable for indicating areas that have escaped atmospheric oxidation
- Iron is one of our biggest potential problems, and we are moving to samples high in Fe to control for this

Detection of phosphate minerals

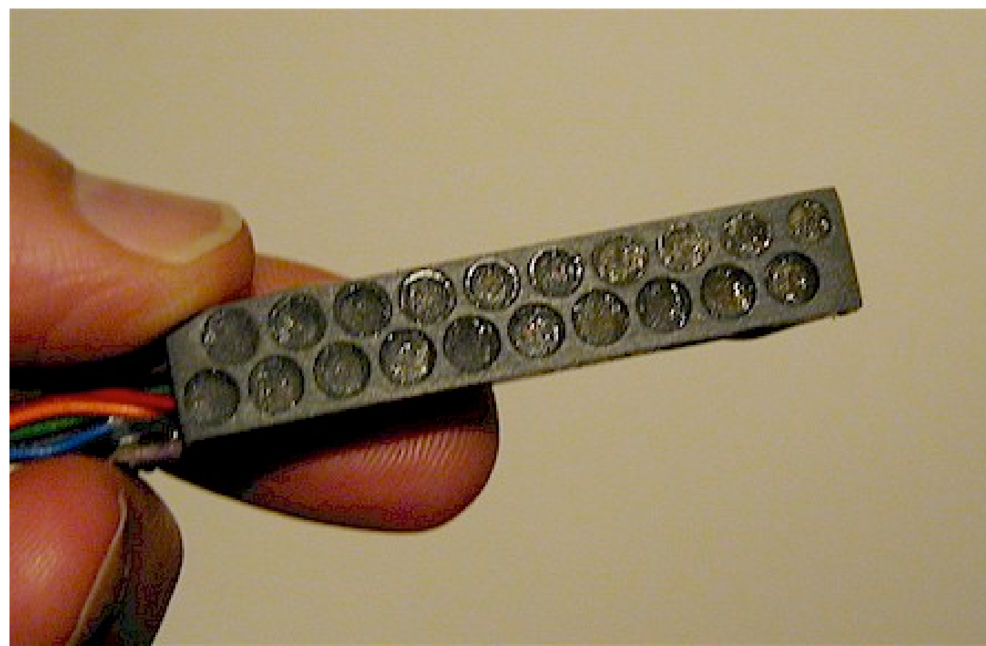


Struvite is a phosphate mineral that, on earth, is an absolute biosignature [$MgNH_3PO_4nH_2O$, where Mg can be replaced by Ca, Cd, UO_2, or AsO_2; NH_3 can be replaced by H, Na, Ce, K, Na, or both K and Na; and n=6 or 14]

Assays for phosphate minerals

- Some are water soluble, such as a new analog just discovered in our group
- Many others are not
- Look for gas evolution in dilute HCl, ammonia release in base, and absorbance spectroscopy
- These techniques will complement APXS

Putting it all together



Final steps

- Do we get the same results with all ISEs on one probe?
- How many extraction systems can we have?
- How many reagents can be added without interference?
- Which of these extraction systems and reagents are the most critical?

Issues

- High amounts of iron on Mars
- Need a pre-extraction unit outside the RCAL that will deliver extracted and filtered soil/crushed rock

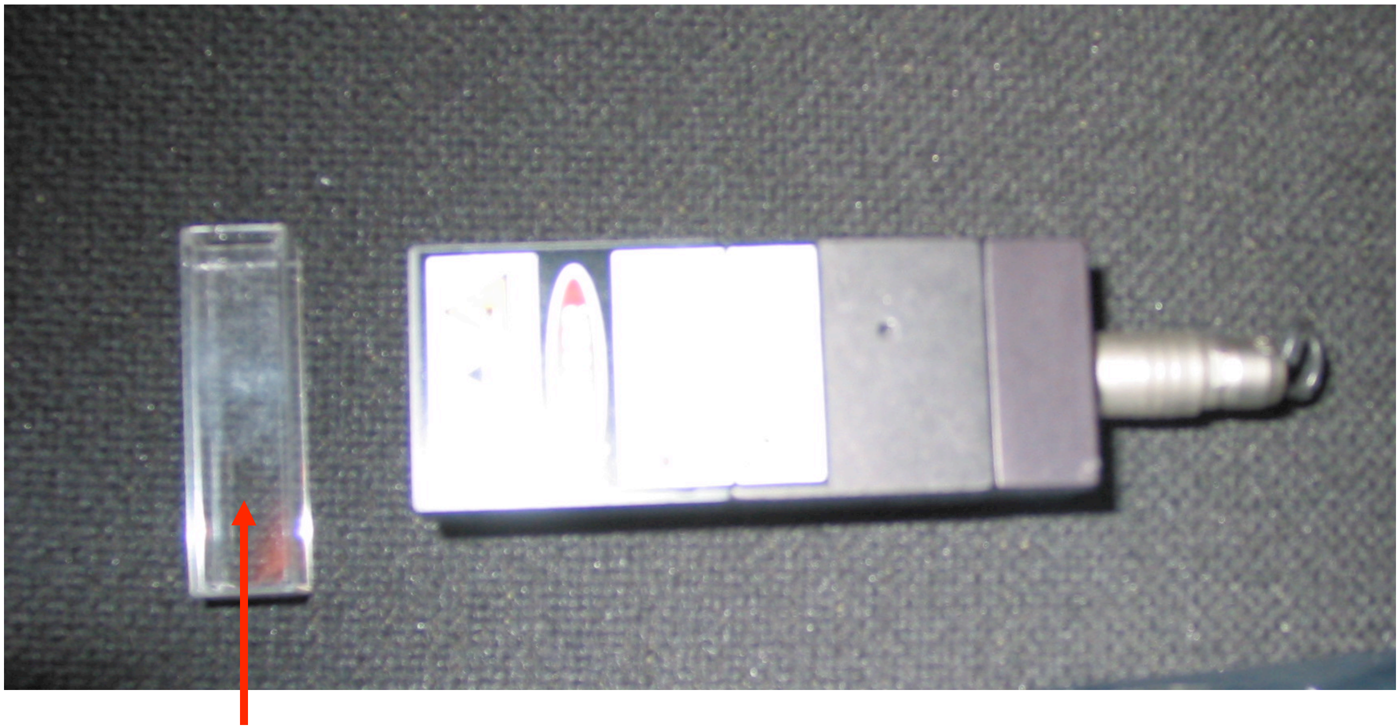
3. Fluorescence tests for organics and life forms

- The ideal: what we wish we could see
- The tools
- Increasing resolution with the tools that we have: biofunctionalization of surfaces
- Specific dyes that yield good signal-to-noise in soil samples
- Work still to be done

"Life detection" on earth

- Wash sample; extract organisms if desired
- Spread onto microscope slide
- Fix and stain
- Inspect by high-power microscopy

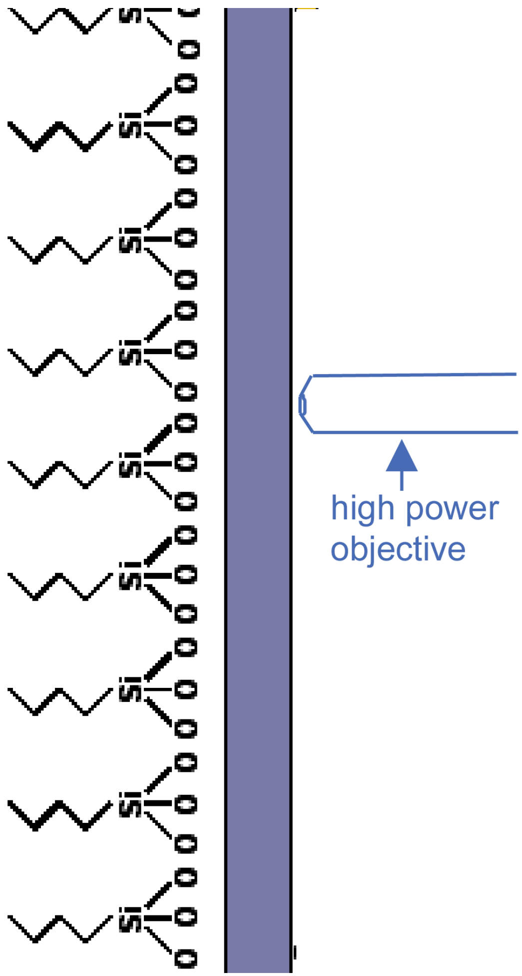
In situ problem



Slurry or filtrate, perhaps containing microbes

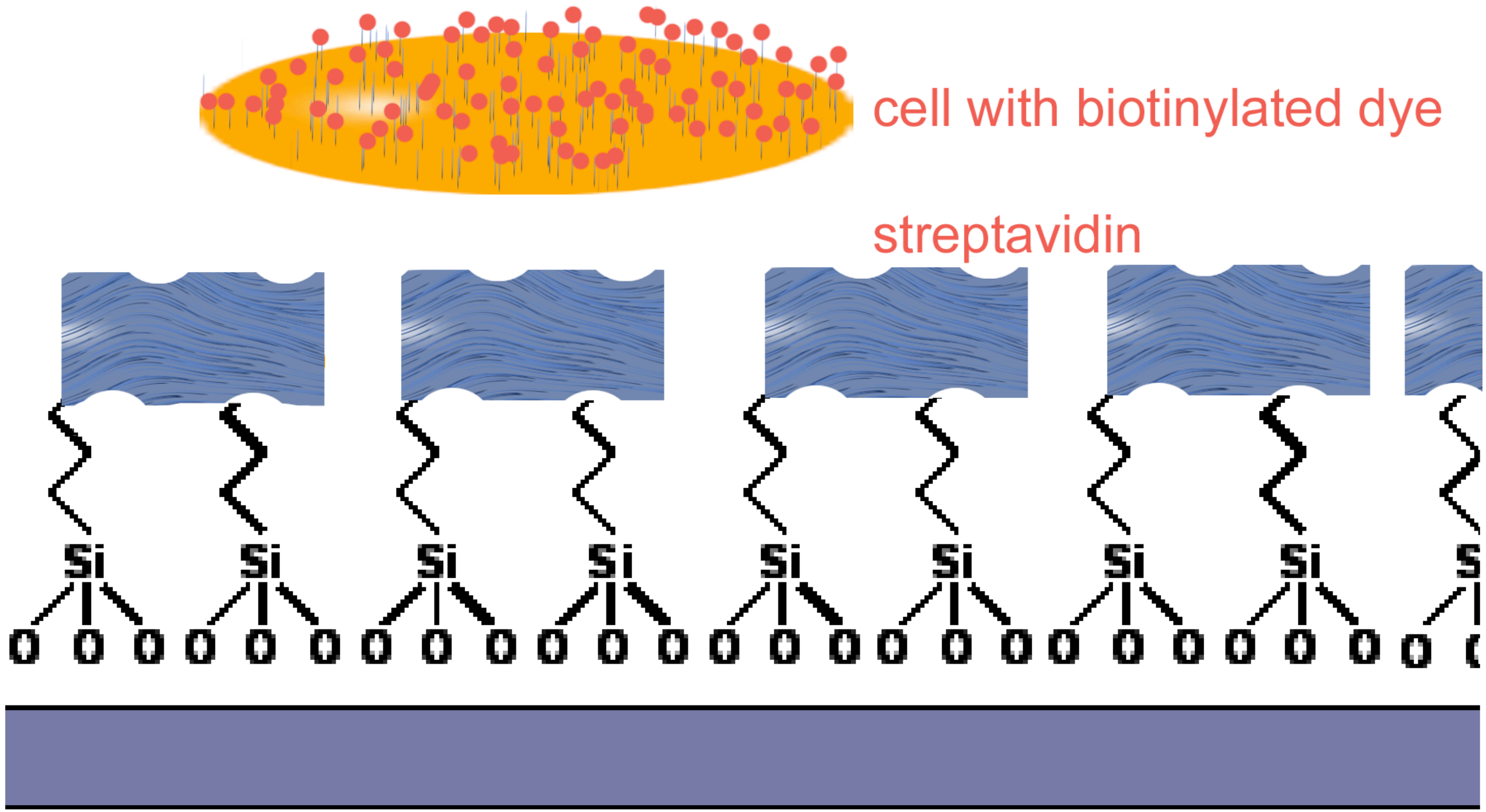
Solution: get the sample closer to the detector!

Biofunctionalization of glass slides

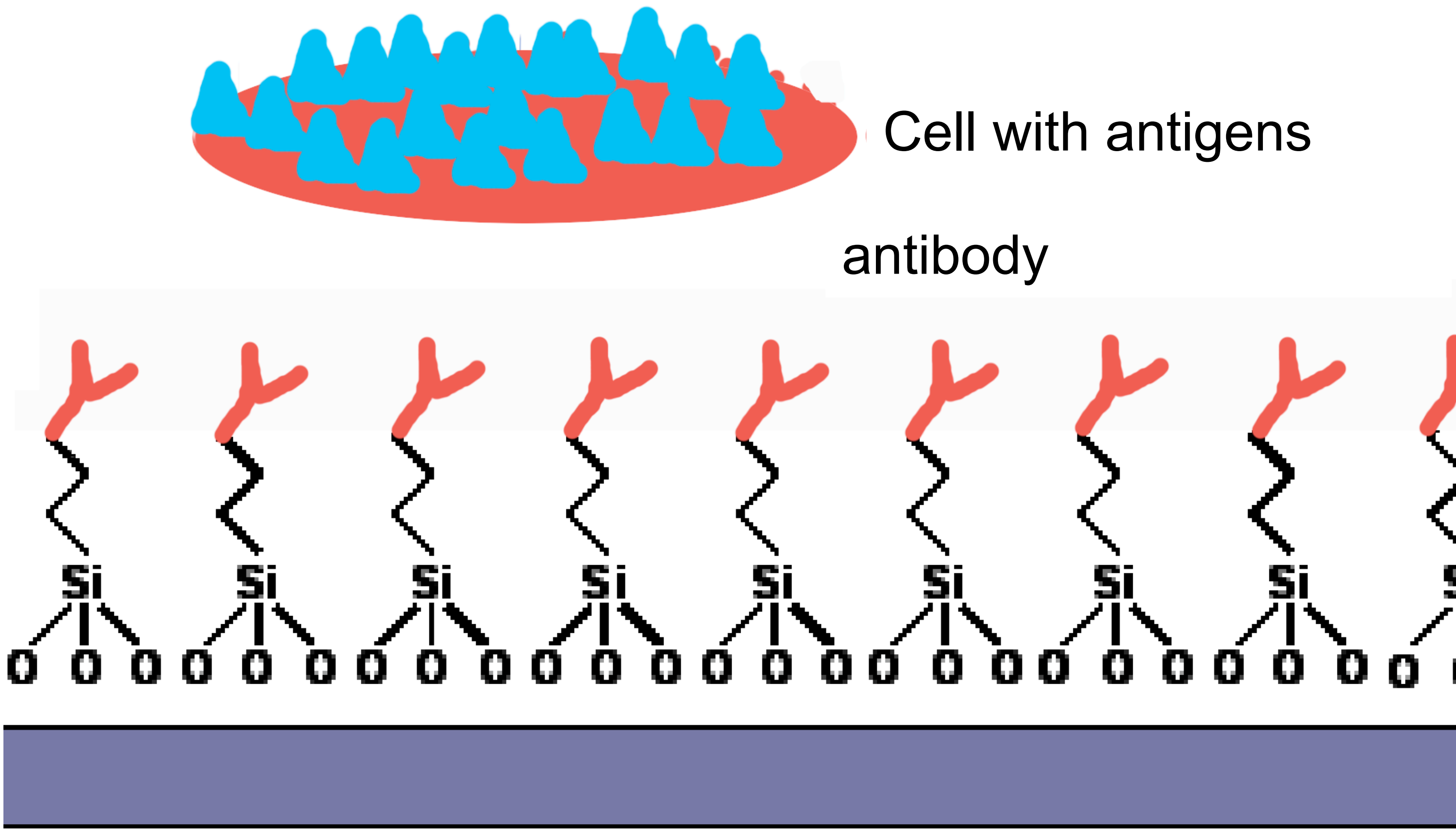


- A silane with a reactive group is attached to the interior walls of the cuvette
- A high-power, short working distance objective can then be used to visualize objects that stick to the surface

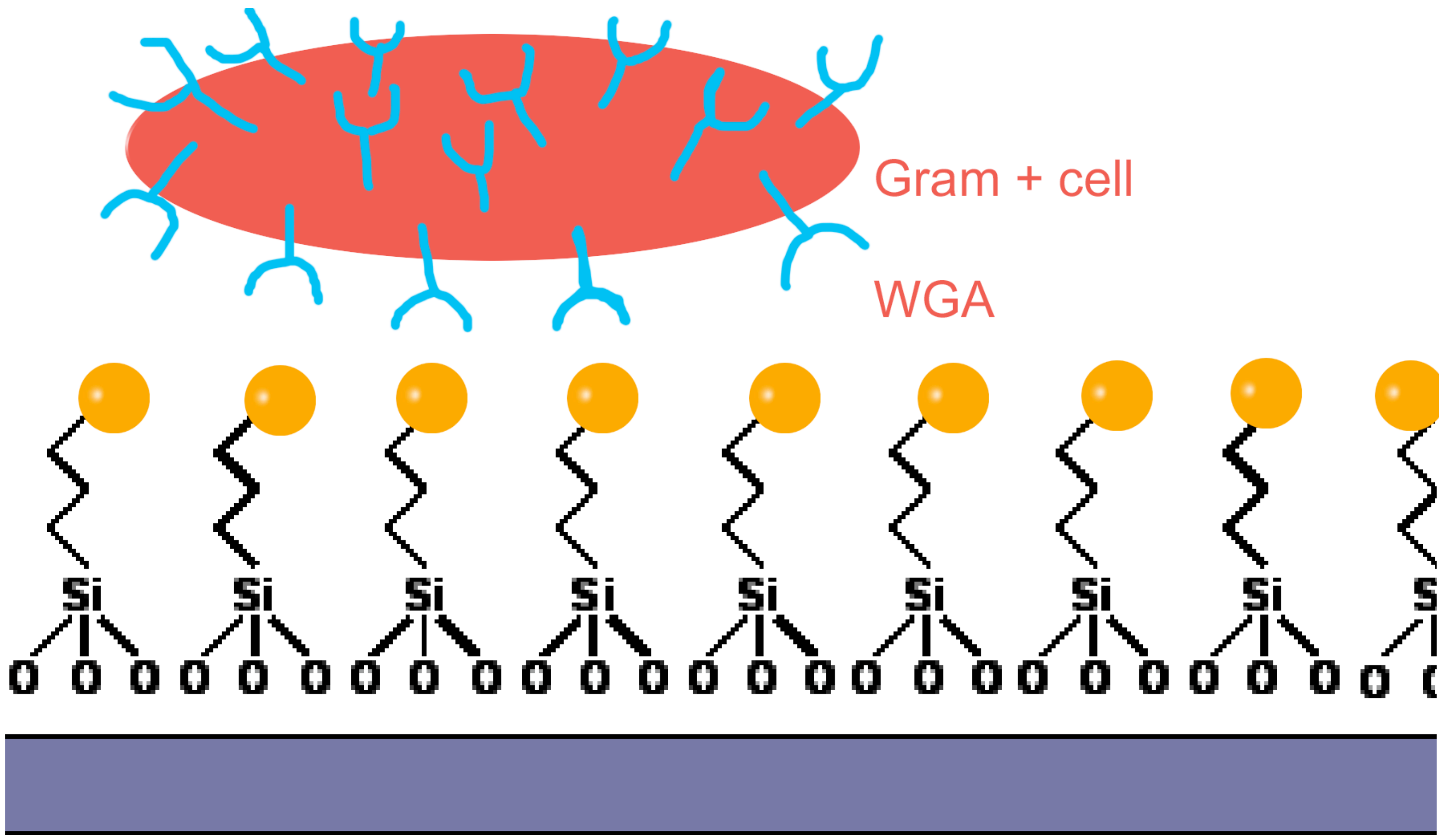
Binding schemes



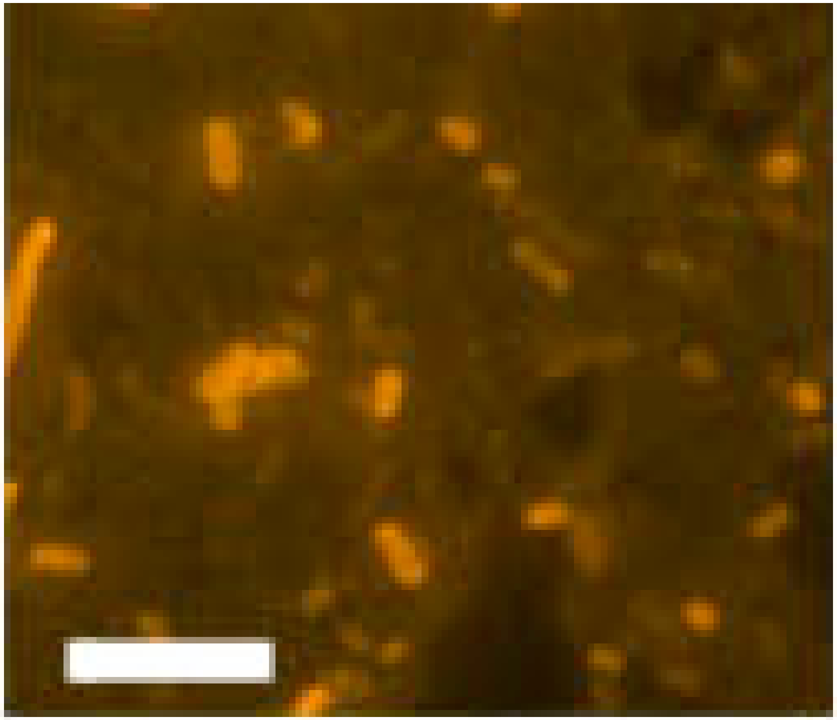
Scheme 2



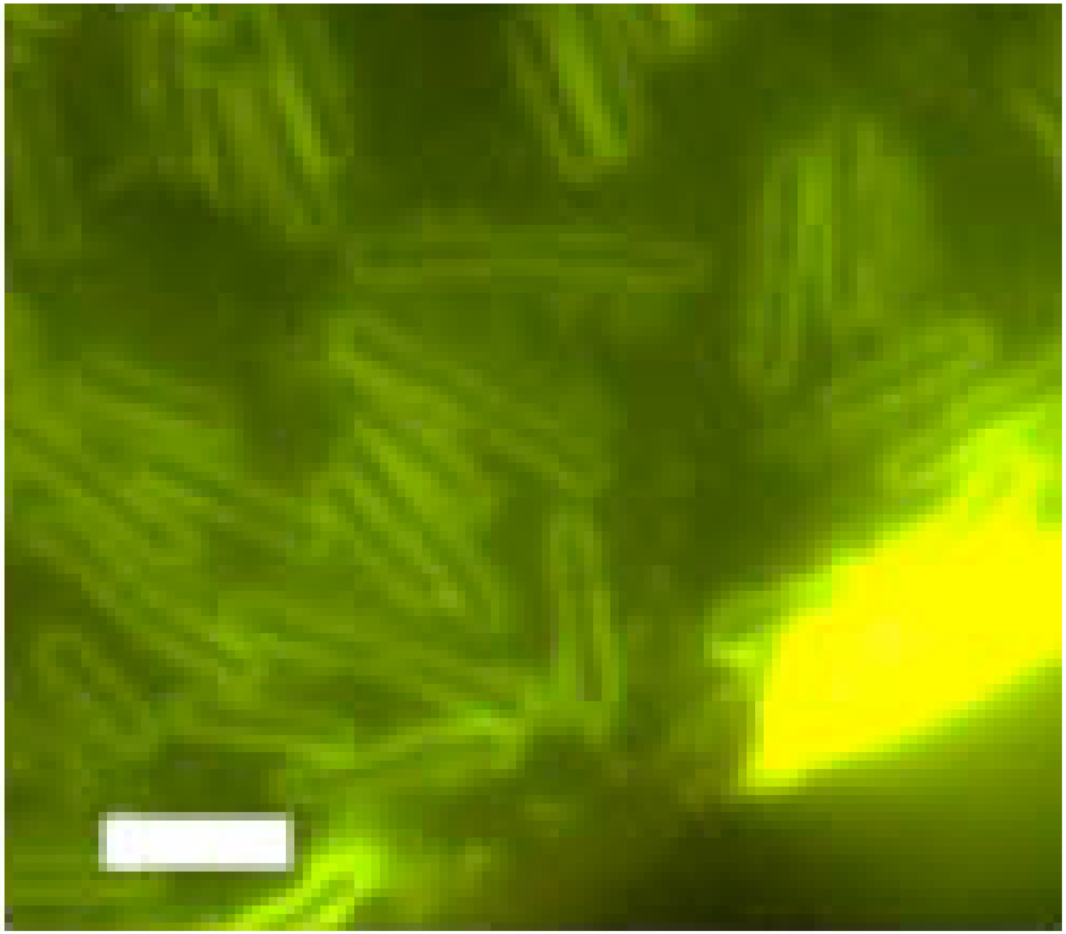
Scheme 3


Gram + cell
WGA
Si
Si
Si
Si
Si
Si
Si
Si
O O O O O O O O O O O O O O O O O O O O O O O O

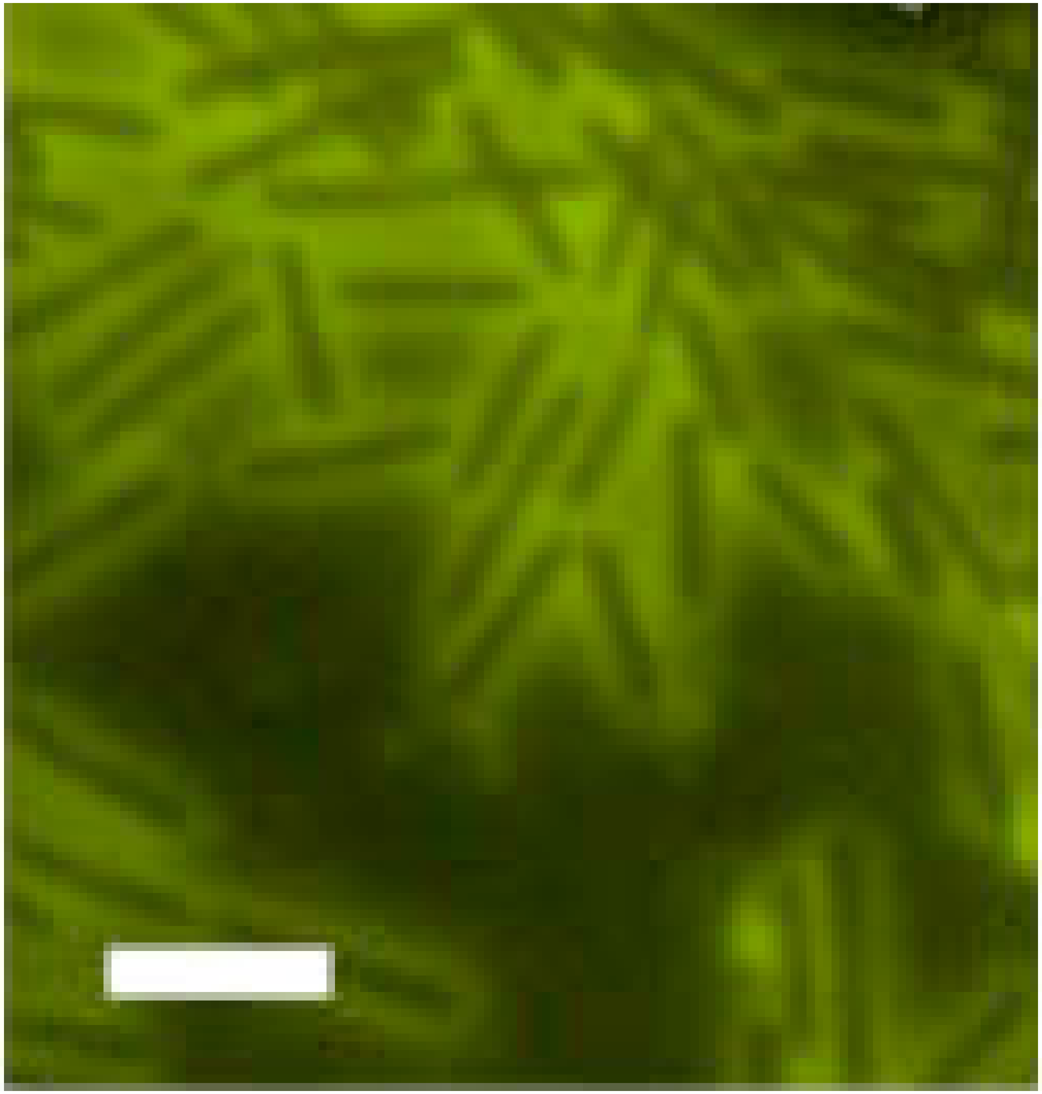
Excellent results obtained with a variety of bacteria and fluorescent probes



E. coli in unfiltered soil
Biotinylated di-4-ANEPPS



Reactive biotin-dye
stuck to *B. subtilis;*
streptavidin functionalization



WGA

B. subtilis

E. coli antibodies

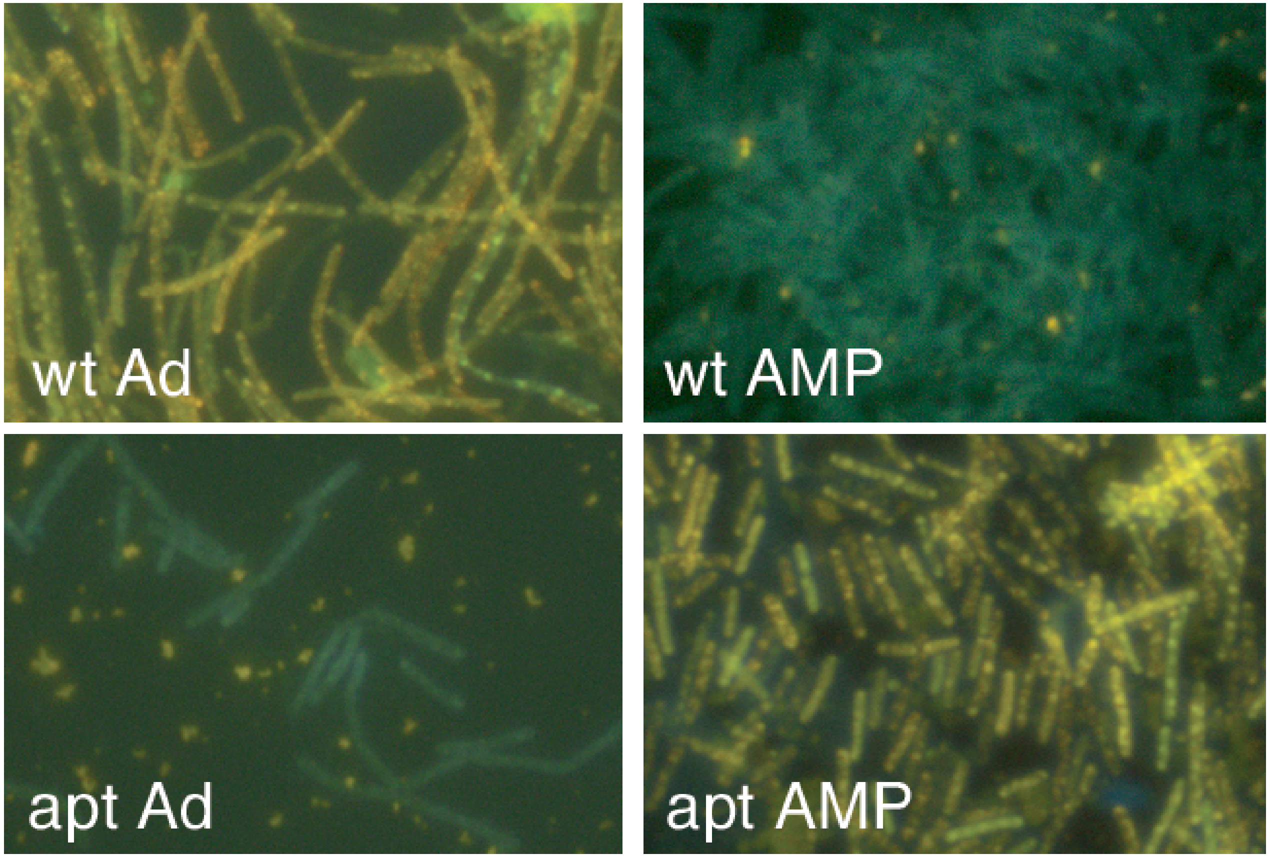
Conclusions

- Biofunctionalization works well
- Sensitivity is 100 cells/mL or fewer!
- Can survive at least 1 yr of freezing
- A robust set of dyes with different Stokes shifts are di-4-ANEPPS, WGA-Alexa (choice of wavelengths), and simply reactive biotin-Alexa
- Of these, di-4-ANEPPS is the most sensitive, with visual and spectroscopic detection of as little as 1 cell or GUV in 1 mL

Issues

- Currently using 405 nm laser; going bluer would permit analysis of some minerals
- Currently using 420 nm longpass out filter
- Biofunctionalization stability must be established

Development of novel QD probes



Low-background, metabolism-specific, extremely stable

Summary and thanks

- We have a wet chemistry suite with greater capabilities than past instruments
- Many of the reagents are essentially weightless and can be chosen at the last minute
- Extraction is the largest issue

- Thanks to: Co-Is: Sam Kounaves, Mike Hecht, Henry Sun, Susanne Douglas; Starsys Research
- EPO team: Marguerite Syvertson; Science